REMARKS

In an office action mailed January 21, 2009, claim 40 has been withdrawn by the Examiner, claims 26, 27, 34 and 39 have been rejected under 35 U.S.C. §102(b), and claims 26-33 and 35-39 have been rejected under 35 U.S.C. §103(a).

In response, Applicant provides the herein remarks. No amendments have been made. Claims 10, 15-17, 19-23 and 40 remain withdrawn. Claims 26-39 are pending examination in the application. Reconsideration is respectfully requested.

Rejections Under §102

Claims 26, 27, 34 and 39 have been rejected under §102(b) as allegedly being anticipated by Fueyo et al., as evidenced by Nevins. According to the Examiner, Fueyo et al. teach a "replication competent" recombinant adenovirus. The Examiner asserts that because Fueyo et al's virus allegedly "induced cell death even in mutant-p53 cells," the virus must be capable of restoring p53 apoptosis in target cells hampered in the p53 apoptosis pathway.

The Examiner contends that the whereby clause in claim 26 ("whereby said restoring factor induces accelerated cell lysis and/or faster release of virus progeny when compared to a recombinant adenovirus lacking said coding sequence") should not be given patentable weight.

The Examiner recognizes that Fueyo et al. do not make a comparison between their adenovirus and a recombinant adenovirus lacking the claimed coding sequence, but alleges that the structure of the Fueyo et al. adenovirus satisfies the structural limitations of the instant claims.

Finally, the Examiner asserts that the p53 derivative disclosed in Fueyo et al. meets the limitations of the claims. According to the Examiner, Fueyo et al. teaches all of the claim limitations and, thus, anticipate the claims. Applicant respectfully disagrees.

In response, Applicant emphasizes that the adenovirus of Fueyo et al. does not include a *coding sequence of at least one mammalian restoring factor functional in restoring the p53 apoptosis pathway*, as required by claim 26.

In addition, the 24 base pair E1A deletion, as disclosed in Fueyo ey al., is not able to bind Rb (see abstract of Fueyo et al., and page 3, right column, lines 3-6). Therefore, expression of this mutant E1A protein will not induce the release of E2F from existing Rb-E2F complexes. The lack of release of E2F, and hence the lack of activation of E2F, will not result in activation of the p53 pathway as evidenced by Nevins.

Therefore, the mutant E1A as disclosed by Fueyo et al. is not able to restore the p53 apoptosis pathway because said protein cannot bind Rb. The reported cell death of mutant p53 cells by the Δ24 adenovirus, as reported by Fueyo et al. is thus not mediated by restoration of the p53-mediated apoptosis pathway.

Accordingly, Fueyo et al. do not disclose an adenovirus comprising a coding sequence of at least one mammalian restoring factor functional in restoring the p53 apoptosis pathway, as claimed. Applicant respectfully requests that the Examiner reconsider and withdraw the above §102 rejection based on Fueyo et al, as evidenced by Nevins.

Accordingly, in light of the above, Applicant respectfully requests that the Examiner reconsider and withdraw the §102(b) rejection based on Fueyo et al. as evidenced by Nevins.

Rejections Under §103

Claims 26-33 and 35-39 have been rejected under §103(a) as being unpatentable over Chang et al. in view of Lin et al. According to the Examiner, Chang et al. and Lin et al. collectively teach most of the structural limitations of the claimed adenovirus.

The Examiner recognizes that Chang et al. do not teach that the target cells are hampered in the p53 dependent apoptosis pathway. The Examiner asserts that because Lin et al. allegedly teach an adenovirus that restores function in calls that lack endogenous p53, it would have been obvious to incorporate the tissue specific replication conditional controls of Chang et al. into the adenovirus p53 construct of Lin et al. and arrive at the instant invention. Applicant respectfully disagrees.

Chang et al. relate to cell-specific viruses, such as conditionally replicating adenoviruses, that transduce cytotoxic genes selected from, for example, thymidine kinase, cytosine deaminase, diphtheria toxin, anti-sense RNA and ribozyme, and cytokines (column 22, lines 16-39).

Lin et al. relate to a replication defective adenovirus that transduces p53 or mutant p53. Lin et al. are not concerned with the effect of p53 on virus replication.
could not have derived the "reasonable expectation of success" required from them.

Neither Lin et al. nor Chang et al. disclose the adenovirus genome comprises a coding sequence of at least one mammalian restoring factor functional in restoring the p53 apoptosis pathway in said target cells.

In order to establish *prima facie* obviousness rejection under §103, one of the criteria to be met is that upon combining the references, all of the claim limitations must be taught.

Applicants have explained the importance of the adenovirus genome comprising a coding sequence of at least one mammalian restoring factor functional in restoring the p53 apoptosis pathway in the target cells. See above.

Upon combining the teachings of Lin et al. and Chang et al., all of the claim limitations re not met. Therefore, Applicants respectfully request that the rejection under §103 based on Lin et al. in view of Chang et al. be reconsidered and withdrawn.

Furthermore, Applicants respectfully submit that the Examiner has used hindsight in combining Lin et al. and Chang et al. At the time of filing the present application, the skilled person had no reasonable expectation of success for such a combination.

The interest in replication competent adenoviruses in the targeting of tumors comes from the fact that such vectors have a better penetration than typical replication defective adenoviruses. It is thought that these viruses are more effective because they release from the cell to infect neighboring cells. This in situ amplification effect is essential; and inherent in the use of replication competent viruses for this purpose (See, Hermiston and Kuhn, first paragraph

of the introduction on page 1022, attached hereto). This review was published shortly after the effective date of the application.

A skilled person considering the development of a novel replication competent virus for this purpose would thus never incorporate the coding region for a protein that would attenuate virus replication. In the mind set of the skilled person, such a protein negates the utility of the replication competent virus. The skilled person would therefore not select such a coding region for incorporation into a replication competent adenovirus with the expectation that such a coding region would increase the effectiveness of the replication competent virus.

This lack of reasonable expectation of success is clearly illustrated on page 1026 of Hermiston and Kuhn, right hand column, which states:

"A second disadvantage of using oncogene inhibitors or tumor supressors to arm replication competent oncolytic viruses is that the action of the inhibitors and supressors, while toxic to the target tumor cell, is also likely to attenuate virus replication."

Furthermore, Applicants respectfully remind the Examiner of his own "expectation of success" expressed in the Office Action dated July 31, 2006. Under §112 on page 6 of the office action, the Examiner interpreted the art and concluded that p53 dependent apoptosis is prevented through the action of the E1B proteins. The Examiner came to the same conclusion as the skilled person at the time of the invention, i.e. that the combination of Lin and Chang would not work.

It is respectfully submitted that the combination of Lin and Chang is only possible using the knowledge of the invention, i.e. hindsight. In fact, it was the present inventors that discovered the surprising effect of the replication competent viruses of the invention. There was no indication of this effect in the art available at the time the application was filed. The available art actually teaches away from the present invention. The negative aspects associated with the viruses of the invention prevented the skilled person from having any reasonable expectation of success.

Again, in light of the foregoing, it is respectfully requested that the Examiner reconsider and withdraw the §103 rejections.

It is now believed that the application is in condition for allowance. If the Examiner believes a telephone discussion would be beneficial to resolve any outstanding issue, he is invited to contact the undersigned without hesitation.

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Review

Armed therapeutic viruses: Strategies and challenges to arming oncolytic viruses with therapeutic genes

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Oncolytic viruses are attractive therapeutics for cancer because they selectively amplify, through replication and spread, the input dose of virus in the target tumor. To date, clinical trials have demonstrated marked safety but have not realized their theoretical efficacy potential. In this review, we consider the potential of armed therapeutic viruses, whose lytic potential is enhanced by genetically engineered therapeutic transgene expression from the virus, as potential vehicles to increase the potency of these agents. Several classes of therapeutic genes are outlined, and potential synergies and hurdles to their delivery from replicating viruses are discussed.

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Keywords: oncolytic virus; armed therapeutic virus; gene therapy; cancer

Tumor-selective, replication-competent oncolytic viruses offer several unique features as cancer therapeutics. First, the input dose is amplified in a tumor-dependent fashion. Consequently, even if only a small proportion of the input dose infects some of the target tumor cells, this infective dose should be capable of replicating in and eliminating neoplastic cells, using successive waves of replication and lysis until the tumor mass is completely destroyed. Importantly, these tumor-selective replication competent viruses spare normal tissue. Because replication-selective oncolytic viruses do not replicate efficiently in normal cells, the associated toxicities should be low. This property will become critical for systemic viral delivery to treat metastatic disease. Low toxicity creates an opportunity for the investigator to increase the dose of the therapeutic virus to overcome losses associated with nonspecific uptake or neutralization due to specific (e.g., antibodies) and nonspecific (e.g., albumin) factors. With their capacity to be carried passively throughout the body via the blood or lymph circulatory systems, these agents should be able to reach, infect, and similarly eliminate all metastatic lesions. These replication-competent, tumor-specific oncolytic viruses offer hope in the daunting field of cancer therapy.

A number of replication-competent, tumor-selective oncolytic viruses have entered the clinic. Clinical experiences show that these agents are safe, but are not potent enough as monotherapies to effect complete tumor regressions or to generate sustained clinical responses. Insufficient or inefficient infection of tumor cells is generally observed. Three strategies are being pursued to overcome this weakness. One is to create less attenuated (more potent) viruses either through use of alternative viruses or by employing alternative, less attenuating, mechanisms for restricting replication to tumor cells.[1–3] The second is to employ additional cytotoxic mechanisms, beyond the direct lytic functions of the virus, by arming these viruses with therapeutic genes.[4] Particularly attractive in this context are those cytotoxic mechanisms with potent bystander effects capable of eliminating tumor cells that the virus cannot reach. And the third is to combine the oncolytic viral therapy with the more traditional radiotherapy and/or chemotherapy, with which virotherapies often synergize.[5]

This review will summarize current clinical results with replication-selective oncolytic viruses (Table 1). We will examine gene therapy strategies using nonreplicating viral vectors, as these inform current strategies for improving oncolytic therapies. Particular focus will be given to strategies for arming oncolytic viruses with therapeutic genes capable of eliciting antitumor immune function, inhibition of tumor neovascularization, or prodrug activation. Through synergistic combination of several cytotoxic modalities (viral lysis, immune or antiangiogenic function, surgery and/or chemo- and radiotherapy), therapies capable of eradicating tumors may be generated.

Oncolytic viruses

Since the early 1900s, reports of tumor regression correlating with either viral vaccination or infection have peaked interest in the oncolytic potential of viruses. The first clinical trial of replicating viruses (using wild-type adenoviruses) was done in 1956.[6] There are suggestions of efficacy in the results of that trial, but lack of understanding of both the disease and

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Table 1 Oncolytic viruses

Viral agent	Genetic alteration	Target tissue or cell pathway	Therapeutic gene	Indication	Stage of clinical development	Reference
Adenoviruses						
ONYX-015 (dl1520)	E1B–55 kDa deletion	p53	–	Head and neck	Phase III	[3,6,104–108]
				Ovarian cancer	Phase I	
				Colorectal cancer	Phases I–II	
				Pancreatic cancer	Phase I	
				Hepatocellular Carcinoma	Phase I Phases I–II	
Ad5-CD/TKrep	E1B–55 kDa deletion	p53	*CD/TK fusion*	Prostate cancer	Phase I	[30,109]
Ad.TK^{rc}(II)	E1B–55 kDa deletion	p53	*TK*	Colon cancer	–	[110]
dl922–947	E1A mutation	Rb pathway	–	Solid tumors	–	[111]
Δ24	E1A mutation	Rb pathway	–	Solid tumors	–	[112,113]
E1Adl01/07	E1A mutation	Proliferating cells	–	Solid tumors	–	[114]
KD1, KD3	E1A mutation	Proliferating cells, immunoprivileged state of tumor	–	Solid tumors	–	[115]
KD1-SPB	E1A mutation/promoter driving E4	Proliferating cells, immunoprivileged state of tumor	–	Lung cancer	–	[116]
CV706	PSA promoter-driven E1A	Prostate	–	Prostate cancer	Phases I–II	[117,118]
CV787	Probasin-driven E1A and PSA-driven E1B	Prostate	–	Prostate cancer	Phases I–II	[119]
vcF11	Tcf4-driven E1A and E4	Colon	–	Colon cancer	–	[120]
ONYX-411	E2F-driven E1A and E4	Rb pathway	–	Solid tumors	–	[1]
AvE1a04i	α-Fetoprotein–driven E1A	Liver	–	Hepatocellular carcinoma	–	[121]
ONYX-304	E3–gp19 kDa deletion	Immunoprivileged state of tumor	*CD*	Solid tumors	–	[89]
ONYX-323	E3-gp19 kDa deletion	Immunoprivileged state of tumor	*TNF*	Solid tumors	–	[89]
IG.Ad5E1(+). E3TK	E3-gp19 kDa deletion	Immunoprivileged state of tumor	*TK*	Solid tumors	–	[96]
AdTyrΔ24, AdTyrΔ2Δ24	Tyrosinase promoter-driven mutant E1A	Melanoma	–	Melanoma	–	[122]

Ad.Flk-1, Ad.Flk-Endo	Flk promoter-driven; E1A±endogilin promoter-driven E1B	Dividing endothelium	–	Solid tumors	–	[53]
01/PEME	p53-responsive promoter-driven E2F antagonist to control E1A and E2A expression	p53	–	Solid tumors	–	[123]
AdE2F-1CRc	E2F promoter-driven E1A	Proliferating cells	–	Solid tumors	–	[124]
AdAFPep/Rep	AFP promoter-driving E1A 13S, E1B–55 kDa deleted	p53	–	Hapatocellular carcinoma	–	[125]
Adl118	E1B deleted	p53	–	Breast cancer	–	[126]
Ad.DF3-E1	DF3/MUC1 promoter-driven E1A	MUC1-positive human carcinomas	*TNF*	Breast cancer	–	[86]
Adp53rc	ADP deletion	Unclear	*p53*	Solid tumors	–	[22]
HSV–derived viruses						
G207	γ34.5 and ICP6 deletion	Proliferating cells, IFN	–	Malignant glioma	Phases I–II	[8,127]
1716	γ34.5 deletion	Proliferating cells, IFN	–	Malignant glioma	Phase I	[9,128]
NV1020 (R7020)	γ34.5 deletion	Proliferating cells, IFN	–	Solid tumors	Phase I	[129]
3616UB	Uracil DNA glycosylase and γ34.5 deletion	Proliferating cells, IFN	–	Solid tumors	–	[130]
M002	γ34.5 deletion	Proliferating cells, IFN	*IL-12*	Solid tumors	–	[131]
Fu-10	γ34.5 and ICP6 deletion, selected for syncytial formation	Proliferating cells, IFN	–	Solid tumors	–	[132]
rRp450	ICP6 deleted	Proliferating cells	*CYP2B1*	Colon cancer	–	[133,134]
hrR3	ICP6 deleted	Proliferating cells	–	Solid tumors	–	[135,136]
dvB7Ig/G207	γ34.5 and ICP6 deletion	Proliferating cells, IFN	Soluble *B7-1*	Solid tumors	–	[137]
G92A	Albumin promoter-driven ICP4	Liver	–	Hepatocellular carcinoma	–	[138]
G47Δ	γ34.5, ICP6, and ICP47 deleted	Proliferating cells, IFN, immunoprivileged state of tumor	–	Solid tumors	–	[137]
dlsp+K	TK deleted	Proliferating cells	–	Solid tumors	–	[139]
R8306	γ34.5 deleted	Proliferating cells, IFN	*IL-4*	Solid tumors	–	[85]
Myb34.5	ICP6 deleted, B-myb promoter driving γ34.5	Proliferating cells, IFN	–	Solid tumors	–	[140]

(continued on next page)

Table 1 (*continued*)

Viral agent	Genetic alteration	Target tissue or cell pathway	Therapeutic gene	Indication	Stage of clinical development	Reference
NV1034	γ34.5 deleted	Proliferating cells, IFN	*GM-CSF*	Solid tumors	–	[87]
NV1042	γ34.5 deleted	Proliferating cells, IFN	*IL-12*	Solid tumors	–	[87]
HSV1yCD	ICP6 deleted	Proliferating cells	*CD*	Solid tumors	–	[141]
	γ34.5 deleted	Proliferating cells, IFN	–	Solid tumors	–	[142]
Newcastle disease virus						
PV701	Passage attenuated	IFN	–	Solid tumors	Phases I–II	[10]
Vaccinia						
Various names	TK deleted	Proliferating cells	–	Solid tumors	–	[143–146]
Vaccinia/GM-CSF RV	TK deleted	Proliferating cells	*GM-CSF*	Melanoma	Phase I	[147]
VvEMAP	TK deleted	Proliferating cells	*EMAP-II*	Melanoma	–	[148]
VV-IL-2	TK deleted	Proliferating cells	*IL-2*	Malignant mesothelioma	Phase I	[149]
VMPPNP	TK deleted	Proliferating cells	*PNP*	Solid tumors	–	[144]
VvCD	TK deleted	Proliferating cells	*CD*	Colon cancer	–	[148]
Various names	TK deleted	Proliferating cells	*B7-1, ICAM-1, LFA-3* alone and altogether in a single agent	Solid tumors		[150] and references therein
VvDD-GFP	TK and VGF deleted	Proliferating cells	–	Solid tumors		[151]
Various names	TK deleted	Proliferating cells	*GM-CSF, IFN-γ, TNFα, IL-1β*, alone and combined	Solid tumors		[145,146]
Reovirus						
Type III	None	IFN	–	Solid tumors	Phase I	[102,152]
Polio virus						
PV1 (RIPO)	IRES substitution	Malignant glioma	–	Solid tumors	–	[153]
Vesicular stomatitis virus						
Indiana strain	None	IFN	–	Solid tumors	–	[103,154]
Measles virus						
MV-Edm	Passage attenuated	IFN	–	Ovarian cancer	Phase I	[155,156]

the viral therapeutic agent prevented the development of this oncolytic system. Since then, several replication-selective oncolytic viruses have been tested extensively in the clinic: ONYX-015, Ad5-CD/TKrep, and CV787 and CV706 (all Ad5-derived); 1716 and G207 (both HSV-1-derived); and PV701 and MTH-68/H (Newcastle disease viruses).[7–14] In the clinical setting, these viruses have been administered by many routes: intratumoral, intravenous, intracranial, and intraperitoneal. Safety has been consistently high, toxicity very low, and only in the case of PV701 has a maximum tolerated dose (MTD) been established.[12] Hundreds of courses of virotherapy have been given with no adverse events attributable to the virotherapy itself. For instance, one patient has received over 30 courses of PV701.[12] Especially encouraging is the observation that where preexisting and acquired neutralizing antibodies to these oncolytic agents have been demonstrated, there has been no correlation between these titers and efficacy.[5,12,15]

To date, however, the clinical experience of these single-agent therapies has fallen short of their theoretical promise. In a few cases, full and relatively durable (up to 31 months) cures have been achieved.[12] However, most patients have not experienced measurable regressions. With ONYX-015, the replication-selective oncolytic virus that has been most extensively tested and optimized in the clinic, only 14% of patients showed objective responses due to treatment.[9] Additionally, maintenance of regressions required continuous dosing. Once virotherapy was discontinued, patients suffered early relapses. However, the patients in these trials (mostly Phase I) had failed multiple previous treatments, including surgery, chemo-, and radiotherapies and, consequently, it is commendable and encouraging in these early trials that even 14% of this group responded. However, the distance between the promise of complete and durable tumor eradication by the oncolytic virus, and the results outlined point to a need for improved virotherapy if this is to become a viable treatment for cancer patients in the clinic. In this review, we will examine strategies to increase the efficacy of oncolytic virotherapy through the addition of therapeutic transgenes to generate what have been termed "armed therapeutic viruses",[4] focusing on therapeutic genes currently being used in nonreplicating and replicating viral-based cancer gene therapies and the methods to control their expression in the context of the replicating virus. The potential synergies and challenges these therapeutic agents may hold for a replication-dependent viral-based therapy will also be discussed.

Armed therapeutic viruses

The experience of the oncologist in the clinic and our clearer understanding of the complexity and plasticity of human solid tumors dictate that combination therapies will need to be employed to generate effective, durable responses for the cancer patient. Armed therapeutic viruses that couple the lytic capability of the virus with the capacity to deliver therapeutic factors (armed therapeutic viruses[4]) to more effectively attack the complexity associated with human tumors,[16] then, is a natural evolution of the oncolytic virus-based therapy. This approach takes advantage of the viruses' ability to selectively replicate and spread in the tumor mass to safely and efficiently deliver therapeutic genes to target tissues where the therapeutic gene products can accumulate at times and to levels that afford maximal patient benefit. Choosing the appropriate gene(s) with which to arm the oncolytic virus to enable it to arrest or eradicate the highly plastic, rapidly evolving tumor is a major question that has no simple answers. As a starting point it will be important to consider the potential interactions of the therapeutic factors with the viral-based therapies as a starting point. Several classes of gene therapy–based therapeutics have been traditionally associated with nonreplicating viral-based gene delivery vehicles (antioncogenes, tumor suppressor genes, prodrug-converting enzymes, antiangiogenic, and immunology-based gene therapies). We will briefly review these "genetic payloads," examining the different factors as candidates for delivery from the oncolytic virus and potential issues surrounding each.

Tumor suppressors and antioncogenes as therapeutic transgenes

The study of cancer molecular biology has led to the discovery of a large variety of oncogenes and tumor suppressors whose aberrant expression or function causes oncogenic transformation. Numerous preclinical studies using replication-defective viruses have shown that restoration of tumor suppressor function, or inhibition of oncogene function, slows tumor growth and/or leads to apoptosis or cancer cell death. The theoretical bases for these virotherapies have been reviewed recently in several articles. One disadvantage of virotherapy strategies based on oncogene inhibition stems from the fact that only infected cells in which the transgene is expressed are killed. No bystander effects due to oncogene inhibition have been observed, i.e., uninfected tumor cells are not killed. Current virotherapy vectors are not efficient enough to insure infection of even the majority — much less all — of the tumor cells, even after intratumoral injection.

A second disadvantage of using oncogene inhibitors or tumor suppressors to arm replication-competent oncolytic viruses is that the action of the inhibitors and suppressors, while toxic to the target tumor cell, is also likely to attenuate viral replication.[20] It may be that restraining expression of the oncogene inhibitor or tumor suppressor therapeutic transgene until late in the viral life cycle, when viral replication is essentially complete, would avoid this counterproductive conflict.[4]

A third possible interference between this class of therapeutic transgenes and the replicating vector encoding them stems from the fact that tumor suppressors and oncogene inhibitors generally affect a number of pathways in the cell, any of which may compromise the engineered or endogenous tumor selectivity mechanism of the oncolytic virus or the viruses' ability to replicate in the target tumor cell. The former would be detrimental to safety, and the latter to efficacy.

However, therapies based on expression of tumor suppressors may be more effective than has been predicted based on their known mechanisms of action. For example, *p53* gene transfer studies have unexpectedly demonstrated that the expression of p53 can trigger a number of events to generate beneficial bystander effects,[21–23] any or all of which may synergize with the viral infection. More recently, investigators engineered the *p53* gene into a replicating adenovirus from which it was expressed to high levels at late times postinfection. Surprisingly, this virus demonstrated enhanced preferential lysis of tumor cells to the exclusion of normal cells.[24] The ability of antioncogenes to synergize with the viral infection remains to be tested.

Prodrug therapies

The efficacy of traditional chemotherapies has been hampered by dose-limiting toxicities to normal cells. Prodrug therapies seek to reduce this toxicity by selectively generating the chemotherapeutic agent at the target tumor site. Such prodrug-based cancer therapies have two basic components: an inactive, nontoxic prodrug and a prodrug-activating enzyme (for a recent list, please see Ref. [15]). In this anticancer strategy, the prodrug can (ideally) be delivered systemically at high doses. The prodrug only becomes cytotoxic when activated by the appropriate enzyme. If the activating enzyme is expressed exclusively in tumor cells, then the prodrug will be activated, or become cytotoxic, only at the site of the target cancer cell. Ideally, once activated, the chemotherapeutic drug leaves the cell in an activated cytotoxic form to kill surrounding tumor cells (local bystander effect). Such bystander effects are particularly important to compensate for the inefficient infection and transduction of tumor cells by currently available vectors. Preclinical demonstrations of bystander effects using various prodrug and activating enzyme combinations have been published. In these studies, tumors composed of as few as 10% of prodrug-expressing cells were fully eradicated, whereas control tumors were not.[25,26] However, the activated drugs' range would ideally be limited enough to restrict it from traveling into and damaging normal tissues. In other words, the active drug should have local bystander effect, but very limited or no distal bystander effect.[27]

To try to ensure tumor cell–specific expression of the prodrug-activating enzymes, investigators have employed a number of methods including 1) intratumoral delivery, 2) tissue- or tumor-specific promoters (e.g., PSA, probasin), and 3) engineering of the relevant transgenes into replication-competent, tumor-selective viral systems under the control of the HCMV promoter[28] or under the control of a native viral promoter.[29] Whereas prodrug-based therapies have been administered using a variety of vectors into various cancers, these therapies have not generated meaningful benefit in the clinical setting, presumably due to the poor distribution of the replication-defective viruses used as delivery vehicles.[30] If this is the limitation to these therapies, replication-competent oncolytic viruses encoding prodrug-activating enzymes may prove to be highly effective as they have been shown to increase levels and distribution of genomically encoded factors over replication-defective viruses.[31]

Thymidine kinase (TK) and cytosine deaminase (CD) and their respective prodrugs [ganciclovir (GCV) and 5-flourocytosine (5-FC), respectively] are the most advanced of the prodrug-based therapies. Most recently, a gene fusion of CD/TK was engineered into a replication-competent, tumor-selective adenovirus and tested in a Phase I clinical trial on locally injectable prostatic tumors. The CD/TK fusion enzyme is a promising improvement for the oncolytic adenovirus because it saves genomic space, which is limited in adenovirus, without losing function.[32] In a 14-patient prostate cancer study, the virus was administered to the patients and, 2 days postinjection, the patients were given GCV and 5-FC, with the GCV and 5-FC dosing continuing for a total of 7 days. Two of 14 patients experienced full tumor regression, and an additional four patients had partial regressions (25–80% reduction in PSA levels). No dose-limiting toxicities were observed, and an MTD could not be reached.[28] These early trials indicate that this treatment, once optimized, may be both effective and safe.

Despite the encouraging initial data, prodrugs whose activated form interferes with DNA replication have been shown to limit the ability of the virus to continue to replicate and spread in the tumor.[33,34] These reductions in viral burst size mitigate the cytolytic potential of these viruses and potentially compromise the full utility of this approach. To avoid interference between the therapeutic effects of direct viral lysis and drug-induced cytotoxicity, other prodrugs less toxic to the virus or more optimized dosing schedules will need to be developed. If this can be achieved, these virotherapies should be able to build upon the already encouraging clinical data being generated around combination therapies with the virus and chemotherapy.[5] As this approach should result in reduced systemic toxicities normally associated with chemotherapy, this treatment may also be combined with other treatment modalities such as radiotherapy or immunotherapy. Much current evidence indicates that combined modalities are considerably more successful in fighting cancer than any of the component monotherapies.[5,26,32,34–40]

Antiangiogenic therapies

Unchecked cell proliferation is a hallmark of human cancers. The continued growth of the tumor, however, is dependent upon an adequate supply of oxygen and nutrients from the blood.[41,42] When tumor growth exceeds the normal blood supply to a tissue or organ, new blood vessel formation must be stimulated from surrounding existing vessels to support continued tumor growth. This process, termed tumor neovascularization (a special form of angiogenesis), consists of multiple steps and includes local degradation of the capillary basement membrane, recruitment and proliferation of endothelial cells, and remodeling and formation of a network of new blood vessels.

Tumor neovascularization is an appealing target for cancer therapeutics for several reasons. First, because neovascularization or angiogenesis is a necessity for tumor growth, antiangiogenics could be applied to any solid tumor, regard-

less of origin and independent of whether it is primary or metastatic disease. Second, because many of the angiogenesis inhibitors are "natural" (endogenous, nonsynthetic), these may be well tolerated by the patient in contrast to traditional chemotherapeutics or small molecules, for example.[43,44] Third, the target proliferating tumor endothelium differs significantly from the normal vascular endothelium in the adult. These differences range from proliferation rates (the normal vascular endothelium is quiescent in the adult, with turnover times measured in hundreds of days[45]) to gene expression profiles.[46] These differences offer potentially valuable targets for therapeutic intervention (see below). Lastly, resistance to angiogenesis inhibitors is less likely to occur. Genetic instability is one of the trademarks of the cancerous cell and is the mechanism responsible for acquisition of drug resistance in cancer cells. In contrast to the cancer cell, the target of angiogenic therapy is a normal, genetically stable endothelial cell stimulated to proliferate and migrate in response to angiogenic stimulus from the tumor. With its genetic stability still intact, the normal endothelial cell is less likely to acquire a mutation conveying therapeutic resistance. Consequently, the development of angiogenesis inhibitors, or inhibitors of tumor neovascularization, has become a broad and active area of cancer research (for recent reviews, see Refs. [47–49]).

More than 40 "natural" (endogenous, nonsynthetic) inhibitors of angiogenesis have been discovered and characterized.[48] The development of these inhibitors as therapeutic agents, however, has been hampered by several factors including manufacturing difficulties, and stability and solubility issues. In addition, the majority of these agents are not directly cytotoxic to tumor cells and so it is likely that these angiogenesis inhibitors would need to be expressed on a continuous basis. Gene therapy offers one potential avenue to address many of these issues. The finding that susceptibility to angiogenesis inhibitors can vary by tumor stage[50] and the recent disappointments of antiangiogenic matrix metalloproteinase inhibitors in the clinic[51] have caused investigators to begin to turn their attention to systems where angiogenesis inhibitors can be combined with standard or experimental cancer therapies.[52,53] In addition, more aggressive antiangiogenic therapies have begun to evolve in which investigators are developing systems to proactively eradicate the neovasculature[54,55] in contrast to arresting its growth. Consequently, it is timely to consider inhibitors of angiogenesis in the context of armed therapeutic viruses (oncolytic viruses encoding therapeutic transgenes). To date, however, replicating viruses encoding antiangiogenic therapeutic genes have not been reported.

Immunotherapy

The immune system is a complex mixture of effector molecules and cells that interact with one another to monitor and maintain the health of the host. Harnessing and targeting this potential into an effective therapy that selectively recognizes and eradicates the cancerous tissue remains a highly sought after, yet elusive, goal. Immunotherapy is based on the concept that there are differences between tumor cells and normal cells that can be detected by the immune system and can serve as targets for immune-mediated eradication of malignant disease. This is a very large and active field of gene therapy research and is at the center of the vast majority of the cancer gene therapy trials currently in the clinic. The use of cytokines, costimulatory molecules, and allogeneic major histocompatibility complex (MHC) molecules; the delivery of tumor antigens to dendritic cells (DCs); and the use of recombinant viruses expressing cancer antigens, alone or in combination with any of the previously described factors, all fall under this broad therapeutic umbrella directed at enhancing immune recognition, killing, and clearance of the target tumor cell.

These various strategies are commonly dependent on antigen-presenting cells (APCs) and cytotoxic T lymphocytes (CTLs). The APC is the sentinel for anomalies in the host. APCs include DCs, mononuclear–phagocytic cells, and activated B lymphocytes, with the DC serving as the target cell of choice for many cancer-based immunotherapies. This is because DCs are the most potent of the APCs, having a high capacity for antigen uptake in their immature form and high levels of MHC class I and II molecules, costimulatory molecules (B7-family), and adhesion molecules (ICAM-1, LFA-3, CD11a,c) in their mature form. These characteristics make them highly efficient at sampling the host environment, presenting antigen, and activating naïve T cells.[56–61] In addition, methods for collecting and growing DCs from hematopoietic precursors have been described[58,62,63] and serve to increase their attractiveness as contributors in a therapeutic strategy.

A robust antitumor CTL response has traditionally been the goal of the immunotherapy approach to cancer treatment. The value of the CTL stems from several factors. First, it is specific. Short peptides, 8–11 amino acids in length, derived from proteasome-digested intracellular proteins are shuttled into the endoplasmic reticulum (ER) by specialized transporters associated with antigen processing (TAP1 and TAP2) where they complex with MHC class I molecules. The MHC class I–peptide complex is consequently transported to the cell surface where it is recognized by the T-cell receptor (TCR) of the CTL. In an oversimplification of a complex process, if the APC has appropriately directed the maturation of a CTL that specifically recognizes a tumor antigen, the CTL will act to destroy the cell by one of two pathways. In the first, the CTL, upon antigen recognition, releases perforin and granzyme B, the perforin acting to create pores in the target cell membrane, which the granzyme penetrates to trigger a caspase-mediated apoptotic cascade.[64,65] An alternative pathway for CTL-mediated target cell killing involves a direct interaction between Fas ligand on the surface of the T lymphocyte and Fas receptor on the target cell, which also leads to caspase activation and apoptotic death of the target cell.[66,67] The cell killing event, then, is independent of other cell types and is, theoretically, long-lived, reducing the chance for reoccurrence of the disease.

How tumors evade recognition and clearance by these potent immune mechanisms remains controversial. Detection of tumor antigen–reactive $CD4^+$ and $CD8^+$ T cells and antibodies directed against a wide variety of tumor-associated gene products in human patients who nonetheless

have measurable cancer adds to the evidence that, like many checkpoints to neoplastic disease, the immune response can be circumvented by the human tumor.[68] Consequently it is important to consider several points when immunostimulatory factors and the immune system are considered in association with the replicating viral agent. First, tumor cells evade, manipulate, and proactively attack immune components in order to survive and proliferate. Evasion of the APC can take several forms. These range from tumor-associated factors that inhibit the differentiation, maturation, and/or function of DCs, e.g., VEGF, IL-6, M-CSF, IL-10, PGE_2, and TGF-β.[69,79] Decreased recognition (e.g., loss of MHC class I molecules, loss of peptide transporters, alterations in proteasome function), function (e.g., decreased levels of TCR signaling pathway proteins CD3ζ, $p56^{lck}$, $p59^{fyn}$, and impaired NF-κB activation), lack of appropriate stimuli (tolerance, clonal deletion), or T-cell survival (e.g., Fas ligand, MUC-1, B7-H1) have all been described as tumor-based mechanisms to evade CTL-mediated eradication.[71–85]

These immune-evasive strategies are daunting, but viral infection may be a key to breaking immune tolerance of tumors. It has been proposed that cancer cells are not detected, or quickly become immunologically tolerated, because they are generally not presented to the immune system in a microenvironment that favors the activation of immune cells. An oncolytic virus, then, is an interesting system to consider as a vehicle to generate a systemic immune response to the target tumor. This is, in part, because it is clear that viruses are highly immunogenic, as measured by high levels of antibody and T cells responses described in the normal population for many of the viruses being considered for development of oncolytic viruses. This suggests that the viral infection has the ability to supply "danger" signals, thought necessary to attract and initiate the DC-mediated process of antigen uptake and presentation that ultimately, in theory, leads to the generation of the tumor-specific CTL response. This is the basis for the use of poxvirus-based vaccines for cancer therapy[86] that are now in various stages of clinical trials. Several oncolytic viruses of Ad and HSV origin are being engineered to encode immunostimulatory cytokines in an attempt to enhance their potential at eliciting a systemic immune response that complements the lytic function of the virus.[87–91]

Oncolytic viruses may also break immune tolerance of tumors by reducing tumor burden (through viral lysis) to a point below which an anti-tumor immune response can be effective. Several studies have indicated that immune dysfunction can be correlated with total tumor burden.[32,81] An additional study has shown that the functional nature of the patient's immune response improved after debulking surgery.[92] Taken together, these studies indicate that lowering tumor burden through virus-induced cell death while stimulating antitumor immune response will increase the probability that a therapeutic systemic immune response will be elicited. Generating such a systemic immune response would be important to destroy metastatic disease.

While theoretically very inviting and well supported by preclinical studies, the ability to harness the immune system to generate long-term therapeutic benefit to the patient has not been realized yet in the clinic. Objective responses have been minimal and clear clinical benefit remains questionable. It should be noted that unlike classical vaccine studies performed prophylactically on healthy subjects, gene therapy–based cancer vaccine trials are faced with the challenge of generating an effective immune response to the target human tumor that has, by the time of its detection and the initiation of treatment, evolved in a variety of strategies to evade immune detection and eradication. It should also be noted that Phase I trials are conducted to determine the toxicity of the agent and are generally performed in late-stage patients who have failed chemotherapy, radiation therapy, and/or surgery. This may not be an ideal population for many of the therapies that require a robust immune response. It is hoped that the safety of these agents might justify offering this treatment to early-stage patients, who are expected to have a better chance of mounting a strong immune-based defense and thereby benefiting from these therapies.

Controlling therapeutic transgene expression from "armed" replicating oncolytic viruses

While it is important to consider the therapeutic factors and how they may synergize with the oncolytic virus to maximize therapeutic benefit, it is equally important to consider how these factors will be genetically engineered into their respective viral genomes and how their expression will be controlled. While packaging of therapeutic genes is generally not an issue for large viruses like HSV (nearly 50% of HSV genes are nonessential for viral replication[93]) and vaccinia (where it is estimated that the virus may be able to package approximately 50 kb of foreign DNA[86]), for smaller viruses like Ad, this is a considerable hurdle. For these viruses, gene delivery must be genomically economical. That is, consideration must be given to delivering as many therapeutic genes as possible from a genome that will only stably accommodate, in the case of Ad, approximately 2 kb of additional DNA beyond the size of the normal genome.[94] One strategy has been to generate multiple genes from a single transcript through the use of internal ribosome entry sites (IRESs),[95,96] which have been successfully employed in replicating viruses.[24,33] A second strategy offered by the replicating virus is to use the endogenous viral gene expression control machinery (promoter/enhancer, polyadenylation, and splice signals) to deliver transgenes and, where possible, to selectively replace an individual viral gene or genes with a therapeutic gene of choice. In this strategy, therapeutic transgene expression should follow the normal kinetics of the endogenous substituted gene. If the expression kinetics of the individual sites is diverse, this should enable investigators to tailor their therapeutic gene expression to levels and times they deem optimal to generate maximal therapeutic benefit. If these substitutions do not alter the remaining surrounding genes in a complex transcription unit and these genes are nonessential to the viral life cycle in the infected tumor cell, the investigator may be able to substitute the remaining genes with additional therapeutic genes. In this fashion, a combination of genes that target totally different aspects of tumor biology (e.g., prodrug-converting enzyme, immunostimulatory) could be incorpo-

rated into a single virus, synergizing with the inherent lytic property of the virus to attack the complexity of the tumor. This type of system has recently been described in the replicating Ad,[29,91,97,98] developed in the nonessential, immunoregulatory E3 region transcription unit.

Native viral promoters offer several advantages as the transgene expression system in the armed therapeutic virus. For example, many of the mechanisms to derive tumor specificity are genetically engineered to be the earliest events (i.e., attachment, penetration, immediate early gene expression) in the viral life cycle or are native to the virus. As the tumor-selective mechanism will dictate whether the viral life cycle is allowed to proceed, viral promoters whose expression follows that gating event will not be expressed in a normal, nontumor cell. Linking therapeutic gene expression to the selectivity of the virus should restrict therapeutic gene expression to the target tumor and should exclude it from occurring in nontarget tissue. This is a very important consideration for a systemically administered oncolytic virus targeted at metastatic disease, where a wide array of cells may be exposed to the agent. Thus, a strategy using endogenous late (in the viral life cycle) promoters offers a level of controlled expression in the oncolytic virus that would not be present if a constitutively active promoter (e.g., HCMV) were used.

Native viral promoters may also offer well-characterized gene expression kinetics[29,91,97] and native viral promoters are optimized for expression in the virally infected cell. With the correct choice of gene insertion sites, it has been shown that a replication-competent virus using a native unmodified viral promoter can achieve levels of therapeutic gene expression superior to those seen with the very strong HCMV promoter/enhancer generated from a replication-incompetent agent.[91]

Tissue- or tumor-specific promoters are also possibilities to convey tumor-specific therapeutic gene expression to the oncolytic virus. However, it is important to note that viral attachment and penetration events have the potential to make the nontarget normal cell appear to be a cancer cell to the tissue- or tumor-specific promoter. For example, the Ad penton protein (essential for penetration of the virus following attachment) interacts with $\alpha(v)$ integrins, and triggers PI 3 kinase activity.[93] The PI 3 kinases are considered an excellent target for cancer-based therapies because they initiate complex signaling cascades that mediate proliferation, differentiation, chemotaxis, and survival.[94,97,98] As this pathway is associated with cancer, it may affect a promoter's ability to discern whether the infected cell is "normal" or "tumor" in origin. This does not exclude using tissue- or tumor-specific promoters but will require careful examination of each promoter in the context of each individual virus for its specificity.

Challenges for armed, replicating, oncolytic virus-based therapies

The mechanisms of each of the various classes of gene-based therapeutics when used as monotherapies may be clear, but their potential interactions within the context of a replicating virus are not easily discerned. These interactions will either synergize to increase, or conflict to decrease, patient benefit. The actions of some therapeutic transgenes may synergize with one viral therapy, while interfering with another. Each combination therapy must be individually evaluated. For example, many of the gene-based therapeutic agents outlined previously in this review also have potential antiviral activities associated with them. In the case of the immunostimulatory factors, it is not only a consideration of the factor and its effect on the viral infection. There is also potential for redundant expression because the normal viral infection itself may stimulate various immunostimulatory factors (e.g., cytokines and chemokines). In this context, even if there is redundancy, the investigator will need to give careful consideration to the levels and duration of this effect before simply dismissing some of these seemingly overlapping, or redundant, factors. As most of the prodrug-converting enzymes are targeted towards DNA integrity and replication, these factors and their incorporation into the viral genome would appear to be a significant challenge, requiring careful consideration of the dosing regimen or control of expression of these factors. In the case of antiangiogenic factors, consideration should be given to whether viral replication will be affected by growth in hypoxic cells. This is not to suggest that these challenges cannot be overcome. Instead, these examples are meant to facilitate thought and discussion on how to overcome these potential hurdles as these therapies make their way towards the clinic, and to point to the fact that each therapeutic will require considerable thought to maximize its potency in the tumor microenvironment in association with the replicating virus.

Conclusion

Human tumors are complex entities that continue to challenge modern medicine to develop more effective cancer therapies. Replication-competent oncolytic viruses, either naturally occurring or genetically engineered, represent a new class of agents being developed and tested in the clinical[3,8,10–12,101,102] and preclinical settings.[103–105] These agents, with their capacity to amplify their dose through replication at the target site, then spread within the tumor to lyse neoplastic cells and decrease the tumor burden, represent unique anticancer therapeutics. It is not clear from past studies or from our current understanding of various potential viral agents which virus (or viruses) will best fulfill the oncolytic goals of sustained replication, exquisite specificity, and robust lytic activity when administered to the human tumor. Consequently, new oncolytic agents based on virus types already in the clinic (e.g., Ad, HSV, Newcastle disease virus, reovirus) or through alternative viruses (e.g., measles, poliovirus, VSV, vaccinia) must be explored. To effectively deal with the complex, heterogeneous nature of the tumor pool, however, the therapeutic transgene expression capacity of these viruses will likely also need to be developed. Armed therapeutic viruses, in which a therapeutic gene(s) is genetically engineered into the virus and dependent upon the continued selective replication of the

virus for expression, represent a very appealing tumor treatment and a novel opportunity to generate a single agent that can attack tumors at multiple levels. In addition, it allows the investigator the flexibility to engineer additional factors into the virus to overcome potential or identified deficiencies of the therapy in the clinical setting. It is important to note that treatment with an armed therapeutic virus does not exclude the use of chemotherapy, radiation, or surgery. To the contrary, as reviewed here, theoretical considerations and clinical trial data strongly support the use of these agents in combination with the viral-based therapy. Consequently, armed therapeutic viruses represent a potentially exciting new treatment paradigm for human cancers.

References

1. Wildner O. Oncolytic viruses as therapeutic agents. *Ann Med.* 2001;33:291–304.
2. Ring CJ. Cytolytic viruses as potential anti-cancer agents. *J Gen Virol.* 2002;83(Pt 3):491–502.
3. Kirn D. Replication-selective oncolytic adenoviruses: virotherapy aimed at genetic targets in cancer. *Oncogene.* 2000; 19:6660–6669.
4. Hermiston T. Gene delivery from replication-selective viruses: arming guided missiles in the war against cancer. *J Clin Invest.* 2000;105:1169–1172.
5. Khuri FR, Nemunaitis J, Ganly I, et al. A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat Med.* 2000;6:879–885.
6. Smith RR, Huebner RJ, Rowe WP, Schatten WE, Thomas LB. Studies on the use of viruses in the treatment of carcinoma of the cervix. *Cancer.* 1956;9:1211–1218.
7. Kirn D. Replication-selective oncolytic adenoviruses: virotherapy aimed at genetic targets in cancer. *Oncogene.* 2000; 19:6660–6669.
8. Reid T, Galanis E, Abbruzzese J, et al. Intra-arterial administration of a replication-selective adenovirus (dl1520) in patients with colorectal carcinoma metastatic to the liver: a phase I trial. *Gene Ther.* 2001;8:1618–1626.
9. Nemunaitis J, Khuri F, Ganly I, et al. Phase II trial of intratumoral administration of ONYX-015, a replication-selective adenovirus, in patients with refractory head and neck cancer. *J Clin Oncol.* 2001;19:289–298.
10. Markert JM, Medlock MD, Rabkin SD, et al. Conditionally replicating herpes simplex virus mutant, G207, for the treatment of malignant glioma: results of a phase I trial. *Gene Ther.* 2000;7:867–874.
11. Rampling R, Cruickshank G, Papanastassiou V, et al. Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma. *Gene Ther.* 2000;7:859–866.
12. Pecora AL, Rizvi N, Cohen GI, et al. Phase I trial of intravenous administration of PV701, an oncolytic virus, in patients with advanced solid cancers. *J Clin Oncol.* 2002;20: 2251–2266.
13. Csatary LK, Moss RW, Beuth J, et al. Beneficial treatment of patients with advanced cancer using a Newcastle disease virus vaccine (MTH-68/H). *Anticancer Res.* 1999;19:635–638.
14. Csatary LK, Bakacs T. Use of Newcastle disease virus vaccine (MTH-68/H) in a patient with high-grade glioblastoma. *JAMA.* 1999;281:1588–1589.
15. Kirn D, Niculesco-Duvaz I, Halden G, Springer CJ. The emerging fields of suicide gene therapy and virotherapy. *Trends Mol Med.* 2002;8:S68–S73.
16. Hermiston T. Fighting fire with fire: attacking the complexity of human tumors with armed therapeutic viruses. *Curr Opin Mol Ther.* 2002;4:334–342.
17. McCormick F. Cancer gene therapy: fringe or cutting edge? *Nat Rev Cancer.* 2001;1:130–141.
18. Wilson DR. Viral-mediated gene transfer for cancer treatment. *Curr Pharmacol Biotechnol.* 2002;3:151–164.
19. Willis AC, Chen X. The promise and obstacle of p53 as a cancer therapeutic agent. *Curr Mol Med.* 2002;2:329–345.
20. Levine A, DiMiao D, Coen DM. *Fields Virology* (Fourth Edition). 2001;1:3–18, 119–132.
21. Nishizaki M, Fujiwara T, Tanida T, et al. Recombinant adenovirus expressing wild-type p53 is antiangiogenic: a proposed mechanism for bystander effect. *Clin Cancer Res.* 1999;5:1015–1023.
22. Buckbinder L, Talbott R, Velasco-Miguel S, et al. Induction of the growth inhibitor IGF-binding protein 3 by p53. *Nature.* 1995;377:646–649.
23. Mueller H. Tumor necrosis factor as an antineoplastic agent: pitfalls and promises. *Cell Mol Life Sci.* 1998;54:1291–1298.
24. Sauthoff H, Pipiya T, Heitner S, et al. Late expression of p53 from a replicating adenovirus improves tumor cell killing and is more tumor cell specific than expression of the adenoviral death protein. *Hum Gene Ther.* 2002 (In press).
25. Moolten FL. Tumor chemosensitivity conferred by inserted herpes thymidine kinase genes: paradigm for a prospective cancer control strategy. *Cancer Res.* 1986;46:5276–5281.
26. Stribbling SM, Friedlos F, Martin J, et al. Regressions of established breast carcinoma xenografts by carboxypeptidase G2 suicide gene therapy and the prodrug CMDA are due to a bystander effect. *Hum Gene Ther.* 2000;11:285–292.
27. Knox RJ. Gene-directed enzyme prodrug therapy (GDEPT) — recognizing the present limitations of gene therapy for the treatment of cancer. *Curr Opin Invest Drugs.* 2001;2:835–838.
28. Freytag SO, Kim JH, Khil MS, et al. Phase I study of replication-competent adenovirus-mediated double suicide gene therapy for local recurrence of prostate cancer. *Proc Am Assoc Cancer Res.* 2002;43 (Abstract 5429).
29. Hawkins LK, Hermiston T. Gene delivery from the E3 region of replicating human adenovirus: evaluation of the E3B region. *Gene Ther.* 2001;8:1142–1148.
30. van Dillen IJ, Mulder NH, Vaalburg W, de Vries EF, Hospers GA. Influence of the bystander effect on HSV-tk/GCV gene therapy. A review. *Curr Gene Ther.* 2002;2: 307–322.
31. Ichikawa T, Chiocca EA. Comparative analyses of transgene delivery and expression in tumors inoculated with a replication-conditional or defective viral vector. *Cancer Res.* 2001;61:5336–5339.
32. Freytag SO, Rogulski KR, Paielli DL, Gilbert JD, Kim JH. A novel three-pronged approach to kill cancer cells selectively: concomitant viral, double suicide gene, and radiotherapy. *Hum Gene Ther.* 1998;9:1323–1333.
33. Wildner O, Morris JC. Therapy of peritoneal carcinomatosis from colon cancer with oncolytic adenoviruses. *J Gene Med.* 2000;2:353–360.
34. McCart JA, Puhlmann M, Lee J, et al. Complex interactions between the replicating oncolytic effect and the enzyme/prodrug effect of vaccinia-mediated tumor regression. *Gene Ther.* 2000;7: 1217–1223.
35. Ichikawa T, Petros WP, Ludeman SM, et al. Intraneoplastic

polymer-based delivery of cyclophosphamide for intratumoral bioconversion by a replicating oncolytic viral vector. *Cancer Res.* 2001;61: 864–868.
36. Lee YJ, Galoforo SS, Battle P, et al. Replicating adenoviral vector-mediated transfer of a heat-inducible double suicide gene for gene therapy. *Cancer Gene Ther.* 2001;8:397–404.
37. Nemunaitis J, Swisher SG, Timmons T, et al. Adenovirus-mediated p53 gene transfer in sequence with cisplatin to tumors of patients with non-small-cell lung cancer. *J Clin Oncol.* 2000;18:609–622.
38. Sanchez-Prieto R, Quintanilla M, Cano A, et al. Carcinoma cell lines become sensitive to DNA-damaging agents by the expression of the adenovirus E1A gene. *Oncogene.* 1996;13:1083–1092.
39. Toyoizumi T, Mick R, Abbas AE, et al. Combined therapy with chemotherapeutic agents and herpes simplex virus type 1 ICP34.5 mutant (HSV-1716) in human non-small cell lung cancer. *Hum Gene Ther.* 1999;10:3013–3029.
40. You L, Yang CT, Jablons DM. ONYX-015 works synergistically with chemotherapy in lung cancer cell lines and primary cultures freshly made from lung cancer patients. *Cancer Res* 2000;60:1009–1013.
41. Folkman J. Tumor angiogenesis. *Adv Cancer Res.* 1985; 43:175–203.
42. Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst.* 1990;82:4–6.
43. Thompson WD, Li WW, Maragoudakis M. The clinical manipulation of angiogenesis: pathology, side-effects, surprises, and opportunities with novel human therapies. *J Pathol.* 2000;190:330–337.
44. Folkman J. Therapeutic angiogenesis in ischemic limbs. *Circulation.* 1998;97:1108–1110.
45. Denekamp J. Vascular attack as a therapeutic strategy for cancer. *Cancer Metastasis Rev.* 1990;9:267–282.
46. St Croix B, Rago C, Velculescu V, et al. Genes expressed in human tumor endothelium. *Science.* 2000;289:1197–1202.
47. Talks KL, Harris AL. Current status of antiangiogenic factors. *Br J Haematol.* 2000;109:477–489.
48. Feldman AL, Libutti SK. Progress in antiangiogenic gene therapy of cancer. *Cancer.* 2000;89:1181–1194.
49. Kleinman HK, Liau G. Gene therapy for antiangiogenesis. *J Natl Cancer Inst.* 2001;93:965–967.
50. Bergers G, Javaherian K, Lo KM, Folkman J, Hanahan D. Effects of angiogenesis inhibitors on multistage carcinogenesis in mice. *Science.* 1999;284:808–812.
51. Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science.* 2002;295:2387–2392.
52. Lund EL, Bastholm L, Kristjansen PE. Therapeutic synergy of TNP-470 and ionizing radiation: effects on tumor growth, vessel morphology, and angiogenesis in human glioblastoma multiforme xenografts. *Clin Cancer Res.* 2000; 6:971–978.
53. Bello L, Carrabba G, Giussani C, et al. Low-dose chemotherapy combined with an antiangiogenic drug reduces human glioma growth *in vivo*. *Cancer Res.* 2001;61:7501–7506.
54. Hood JD, Bednarski M, Frausto R, et al. Tumor regression by targeted gene delivery to the neovasculature. *Science.* 2002; 296:2404–2407.
55. Savontaus MJ, Sauter BV, Huang TG, Woo SL. Transcriptional targeting of conditionally replicating adenovirus to dividing endothelial cells. *Gene Ther.* 2002;9:972–979.
56. Steinman RM. The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol.* 1991;9:271–296.
57. Steinman RM. Dendritic cells and immune-based therapies. *Exp Hematol.* 1996;24:859–862.
58. Steinman RM, Pack M, Inaba K. Dendritic cell development and maturation. *Adv Exp Med Biol.* 1997;417:1–6.
59. Lanzavecchia A. Identifying strategies for immune intervention. *Science.* 1993;260:937–944.
60. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature.* 1998;392:245–252.
61. Dallal RM, Lotze MT. Immunotherapy of metastasis. *Surg Oncol Clin North Am.* 2001;10:433–447, xi.
62. Caux C, Massacrier C, Vanbervliet B, et al. $CD34^+$ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to granulocyte–macrophage colony-stimulating factor plus tumor necrosis factor alpha: II. Functional analysis. *Blood.* 1997;90:1458–1470.
63. Young JW, Szabolcs P, Moore MA. Identification of dendritic cell colony-forming units among normal human $CD34^+$ one marrow progenitors that are expanded by c-kit-ligand and yield pure dendritic cell colonies in the presence of granulocyte/macrophage colony-stimulating factor and tumor necrosis factor alpha. *J Exp Med.* 1995;182:1111–1119.
64. Chinnaiyan AM, Hanna WL, Orth K, et al. Cytotoxic T-cell–derived granzyme B activates the apoptotic protease ICE-LAP3. *Curr Biol.* 1996; 6:897–899.
65. Froelich CJ, Dixit VM, Yang X. Lymphocyte granule–mediated apoptosis: matters of viral mimicry and deadly proteases. *Immunol Today.* 1998;19:30–36.
66. Alderson MR, Tough TW, Davis-Smith T, et al. Fas ligand mediates activation-induced cell death in human T lymphocytes. *J Exp Med.* 1995;181: 71–77.
67. Lynch DH, Alderson MR, Ramsdell F. Immunoregulatory effects of Fas-mediated signalling. *J Cell Biochem.* 1996;60: 39–46.
68. Dranoff G. Tumor immunology: immune recognition and tumor protection. *Curr Opin Immunol.* 2002;14:161–164.
69. Vicari AP, Caux C. Chemokines in cancer. *Cytokine Growth Factor Rev.* 2002;13:143–154.
70. Vicari AP, Caux C, Trinchieri G. Tumour escape from immune surveillance through dendritic cell inactivation. *Semin Cancer Biol.* 2002;12:33–42.
71. Hellstrom I, Hellstrom KE. Tumor immunology: an overview. *Ann NY Acad Sci.* 1993;690:24–33.
72. Hellstrom KE, Hellstrom I, Linsley P, Chen L. On the role of costimulation in tumor immunity. *Ann NY Acad Sci.* 1993;690:225–230.
73. Nakagomi H, Petersson M, Magnusson I, et al. Decreased expression of the signal-transducing zeta chains in tumor-infiltrating T-cells and NK cells of patients with colorectal carcinoma. *Cancer Res.* 1993; 53:5610–5612.
74. Finke JH, Zea AH, Stanley J, et al. Loss of T-cell receptor zeta chain and p56lck in T-cells infiltrating human renal cell carcinoma. *Cancer Res.* 1993;53:5613–5616.
75. Ferrone S, Marincola FM. Loss of HLA class I antigens by melanoma cells: molecular mechanisms, functional significance and clinical relevance. *Immunol Today.* 1995;16:487–494.
76. Seliger B, Maeurer MJ, Ferrone S. TAP off-tumors on. *Immunol Today.* 1997;18:292–299.
77. Hersey P. Impediments to successful immunotherapy. *Pharmacol Ther.* 1999;81:111–119.
78. Morford LA, Elliott LH, Carlson SL, Brooks WH, Roszman TL. T cell receptor–mediated signaling is defective in T cells obtained from patients with primary intracranial tumors. *J Immunol.* 1997;159:4415–4425.
79. Sogn JA. Tumor immunology: the glass is half full. *Immunity.* 1998;9:757–763.

80. Ling W, Rayman P, Uzzo R, et al. Impaired activation of NFkappaB in T cells from a subset of renal cell carcinoma patients is mediated by inhibition of phosphorylation and degradation of the inhibitor, IkappaBalpha. *Blood*. 1998;92: 1334–1341.
81. Drake CG, Pardoll DM. Tumor immunology — towards a paradigm of reciprocal research. *Semin Cancer Biol*. 2002; 12:73–80.
82. Pardoll DM. Spinning molecular immunology into successful immunotherapy. *Nat Rev Immunol*. 2002;2:227–238.
83. O'Connell J, Bennett MW, O'Sullivan GC, Collins JK, Shanahan F. Resistance to Fas (APO-1/CD95)–mediated apoptosis and expression of Fas ligand in esophageal cancer: the Fas counterattack. *Dis Esophagus*. 1999; 12:83–89.
84. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med*. 2002;8:793–800.
85. Gimmi CD, Morrison BW, Mainprice BA, et al. Breast cancer-associated antigen, DF3/MUC1, induces apoptosis of activated human T cells. *Nat Med*. 1996;2:1367–1370.
86. Moss B. Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety. *Proc Natl Acad Sci USA*. 1996;93:11341–11348.
87. Andreansky S, He B, van Cott J, et al. Treatment of intracranial gliomas in immunocompetent mice using herpes simplex viruses that express murine interleukins. *Gene Ther*. 1998;5:121–130.
88. Kurihara T, Brough DE, Kovesdi I, Kufe DW. Selectivity of a replication-competent adenovirus for human breast carcinoma cells expressing the MUC1 antigen. *J Clin Invest*. 2000; 106:763–771.
89. Wong RJ, Patel SG, Kim S, et al. Cytokine gene transfer enhances herpes oncolytic therapy in murine squamous cell carcinoma. *Hum Gene Ther*. 2001;12:253–265.
90. Bennett JJ, Malhotra S, Wong RJ, et al. Interleukin 12 secretion enhances antitumor efficacy of oncolytic herpes simplex viral therapy for colorectal cancer. *Ann Surg*. 2001;233:819–826.
91. Hawkins LK, Johnson L, Bauzon M, et al. Gene delivery from the E3 region of replicating human adenovirus: evaluation of the 6.7 K/gp19 K region. *Gene Ther*. 2001;8:1123–1131.
92. Heriot AG, Marriott JB, Cookson S, Kumar D, Dalgleish AG. Reduction in cytokine production in colorectal cancer patients: association with stage and reversal by resection. *Br J Cancer*. 2000;82:1009–1012.
93. Roizman B. The function of herpes simplex virus genes: a primer for genetic engineering of novel vectors. *Proc Natl Acad Sci USA*. 1996;93:11307–11312.
94. Kiessling R, Wasserman S, Horiguchi S, et al. Tumor-induced immune dysfunction. *Cancer Immunol Immunother*. 1999;48:353–362.
95. Li E, Stupack D, Bokuch GM, Nemerow GR. Adenovirus endocytosis requires actin cytoskeleton reorganization mediated by Rho family GTPases. *J Virol*. 1998;72:8806–8812.
96. Roymans D, Slegers H. Phosphatidylinositol 3-kinases in tumor progression. *Eur J Biochem*. 2001;268:487–498.
97. Hawkins LK, Hermiston TW. Gene delivery from the E3 region of replicating human adenovirus: evaluation of the ADP region. *Gene Ther*. 2001;8:1132–1141.
98. Nanda D, Vogels R, Havenga M, et al. Treatment of malignant gliomas with a replicating adenoviral vector expressing herpes simplex virus-thymidine kinase. *Cancer Res*. 2001;61:8743–8750.
99. Katso R, Okkenhaug K, Ahmadi K, et al. Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. *Annu Rev Cell Dev Biol*. 2001;17:615–675.
100. Berrie CP. Phosphoinositide 3-kinase inhibition in cancer treatment. *Expert Opin Invest Drugs*. 2001;10:1085–1098.
101. Hawkins LK, Lemoine NR, Kirn D. Oncolytic biotherapy: a novel therapeutic platform. *Lancet Oncol*. 2002;3:17–26.
102. Kirn D, Martuza RL, Zwiebel J. Replication-selective virotherapy for cancer: biological principles, risk management and future directions. *Nat Med*. 2001;7:781–787.
103. Timiryasova TM, Li J, Chen B, et al. Antitumor effect of vaccinia virus in glioma model. *Oncol Res*. 1999;11:133–144.
104. Coffey MC, Strong JE, Forsyth PA, Lee PW. Reovirus therapy of tumors with activated Ras pathway. *Science*. 1998;282:1332–1334.
105. Stojdl DF, Lichty B, Knowles S, et al. Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. *Nat Med*. 2000;6:821–825.
106. Ganly I, Kirn D, Eckhardt G, et al. A phase I study of Onyx-015, an E1B attenuated adenovirus, administered intratumorally to patients with recurrent head and neck cancer. *Clin Cancer Res*. 2000; 6:798–806.
107. Vasey PA, Shulman LN, Campos S, et al. Phase I trial of intraperitoneal injection of the E1B–55-kd-gene–deleted adenovirus ONYX-015 (dl1520) given on days 1 through 5 every 3 weeks in patients with recurrent/refractory epithelial ovarian cancer. *J Clin Oncol*. 2002;20:1562–1569.
108. Bischoff JR, Kirn DH, Williams A, et al. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science*. 1996;274:373–376.
109. Mulvihill S, Warren R, Venook A, et al. Safety and feasibility of injection with an E1B–55 kDa gene–deleted, replication-selective adenovirus (ONYX-015) into primary carcinomas of the pancreas: a phase I trial. *Gene Ther*. 2001;8:308–315.
110. Habib N, Salama H, Abd El Latif Abu Median A, et al. Clinical trial of E1B-deleted adenovirus (dl1520) gene therapy for hepatocellular carcinoma. *Cancer Gene Ther*. 2002;9:254–259.
111. Freytag SO, Khil M, Stricker H, et al. Phase I study of replication-competent adenovirus-mediated double suicide gene therapy for the treatment of locally recurrent prostate cancer. *Cancer Res*. 2002;62:4968–4976.
112. Wildner O, Blaese RM, Morris JC. Therapy of colon cancer with oncolytic adenovirus is enhanced by the addition of herpes simplex virus-thymidine kinase. *Cancer Res*. 1999;59: 410–413.
113. Heise C, Hermiston T, Johnson L, et al. An adenovirus E1A mutant that demonstrates potent and selective systemic antitumoral efficacy. *Nat Med*. 2000;6:1134–1139.
114. Fueyo J, Gomez-Manzano C, Alemany R, et al. A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect *in vivo*. *Oncogene*. 2000;19:2–12.
115. Suzuki K, Fueyo J, Krasnykh V, et al. A conditionally replicative adenovirus with enhanced infectivity shows improved oncolytic potency. *Clin Cancer Res*. 2001;7:120–126.
116. Howe JA, Demers GW, Johnson DE, et al. Evaluation of E1-mutant adenoviruses as conditionally replicating agents for cancer therapy. *Mol Ther*. 2000;2:485–495.
117. Doronin K, Toth K, Kuppuswamy M, et al. Tumor-specific, replication-competent adenovirus vectors overexpressing the adenovirus death protein. *J Virol*. 2000;74:6147–6155.
118. Doronin K, Kuppuswamy M, Toth K, et al. Tissue-specific, tumor-selective, replication-competent adenovirus vector for cancer gene therapy. *J Virol*. 2001;75:3314–3324.

119. DeWeese TL, van der Poel H, Li S, et al. A phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy. *Cancer Res.* 2001;61: 7464–7472.
120. Chen Y, DeWeese T, Dilley J, et al. CV706, a prostate cancer-specific adenovirus variant, in combination with radiotherapy produces synergistic antitumor efficacy without increasing toxicity. *Cancer Res.* 2001;61:5453–5460.
121. Yu DC, Chen Y, Dilley J, et al. Antitumor synergy of CV787, a prostate cancer-specific adenovirus, and paclitaxel and docetaxel. *Cancer Res.* 2001;61:517–525.
122. Fuerer C, Iggo R. Adenoviruses with Tcf binding sites in multiple early promoters show enhanced selectivity for tumour cells with constitutive activation of the wnt signalling pathway. *Gene Ther.* 2002;9:270–281.
123. Johnson L, Shen A, Boyle L, et al. Selectively replicating adenoviruses targeting deregulated E2F activity are potent, systemic antitumor agents. *Cancer Cell.* 2002;1:325–337.
124. Hallenbeck PL, Chang YN, Hay C, et al. A novel tumor-specific replication-restricted adenoviral vector for gene therapy of hepatocellular carcinoma. *Hum Gene Ther.* 1999; 10:1721–1733.
125. Nettelbeck DM, Rivera AA, Balague C, Alemany R, Curiel DT. Novel oncolytic adenoviruses targeted to melanoma: specific viral replication and cytolysis by expression of E1A mutants from the tyrosinase enhancer/promoter. *Cancer Res.* 2002;62:4663–4670.
126. Ramachandra M, Rahman A, Zou A, et al. Re-engineering adenovirus regulatory pathways to enhance oncolytic specificity and efficacy. *Nat Biotechnol.* 2001;19:1035–1041.
127. Tsukuda K, Wiewrodt R, Molnar-Kimber K, Jovanovic VP, Amin KM. An E2F-responsive replication-selective adenovirus targeted to the defective cell cycle in cancer cells: potent antitumoral efficacy but no toxicity to normal cell. *Cancer Res.* 2002;62:3438–3447.
128. Takahashi M, Sato T, Sagawa T, et al. E1B–55K–deleted adenovirus expressing E1A-13S by AFP-enhancer/promoter is capable of highly specific replication in AFP-producing hepatocellular carcinoma and eradication of established tumor. *Mol Ther.* 2002;5:627–634 (Part 1).
129. Fabra A, Parada C, Vinyals A, et al. Intravascular injections of a conditional replicative adenovirus (adl118) prevent metastatic disease in human breast carcinoma xenografts. *Gene Ther.* 2001;8: 1627–1634.
130. Mineta T, Rabkin SD, Yazaki T, Hunter WD, Martuza RL. Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. *Nat Med.* 1995;1:938–943.
131. MacLean AR, ul-Fareed M, Robertson L, Harland J, Brown SM. Herpes simplex virus type 1 deletion variants 1714 and 1716 pinpoint neurovirulence-related sequences in Glasgow strain 17^+ between immediate early gene 1 and the "a" sequence. *J Gen Virol.* 1991;72:631–639.
132. Delman KA, Bennett JJ, Zager JS, et al. Effects of preexisting immunity on the response to herpes simplex–based oncolytic viral therapy. *Hum Gene Ther.* 2000;11:2465–2472.
133. Pyles RB, Warnick RE, Chalk CL, Szanti BE, Parysek LM. A novel multiply-mutated HSV-1 strain for the treatment of human brain tumors. *Hum Gene Ther.* 1997; 8:533–544.
134. Parker LP, Wolf JK, Price JE. Adenoviral-mediated gene therapy with Ad5CMVp53 and Ad5CMVp21 in combination with standard therapies in human breast cancer cell lines. *Ann Clin Lab Sci.* 2000;30:395–405.
135. Fu X, Zhang X. Potent systemic antitumor activity from an oncolytic herpes simplex virus of syncytial phenotype. *Cancer Res.* 2002;62:2306–2312.
136. Chase M, Chung RY, Chiocca EA. An oncolytic viral mutant that delivers the CYP2B1 transgene and augments cyclophosphamide chemotherapy. *Nat Biotechnol.* 1998;16: 444–448.
137. Pawlik TM, Nakamura H, Mullen JT, et al. Prodrug bioactivation and oncolysis of diffuse liver metastases by a herpes simplex virus 1 mutant that expresses the CYP2B1 transgene. *Cancer.* 2002;95: 1171–1181.
138. Mineta T, Rabkin SD, Martuza RL. Treatment of malignant gliomas using ganciclovir-hypersensitive, ribonucleotide reductase-deficient herpes simplex viral mutant. *Cancer Res.* 1994;54:3963–3966.
139. Boviatsis EJ, Park JS, Sena-Esteves M, et al. Long-term survival of rats harboring brain neoplasms treated with ganciclovir and a herpes simplex virus vector that retains an intact thymidine kinase gene. *Cancer Res.* 1994;54:5745–5751.
140. Todo T, Martuza RL, Rabkin SD, Johnson PA. Oncolytic herpes simplex virus vector with enhanced MHC class I presentation and tumor cell killing. *Proc Natl Acad Sci USA.* 2001;98:6396–6401.
141. Miyatake S, Iyer A, Martuza RL, Rabkin SD. Transcriptional targeting of herpes simplex virus for cell-specific replication. *J Virol.* 1997;71:5124–5132.
142. Martuza RL, Malick A, Markert JM, Ruffner KL, Coen DM. Experimental therapy of human glioma by means of a genetically engineered virus mutant. *Science.* 1991;252:854–856.
143. Chung RY, Saeki Y, Chiocca EA. B-myb promoter retargeting of herpes simplex virus gamma34.5 gene-mediated virulence toward tumor and cycling cells. *J Virol.* 1999;73: 7556–7564.
144. Nakamura H, Mullen JT, Chandrasekhar S, et al. Multimodality therapy with a replication-conditional herpes simplex virus 1 mutant that expresses yeast cytosine deaminase for intratumoral conversion of 5-fluorocytosine to 5-fluorouracil. *Cancer Res.* 2001;61: 5447–5452.
145. Markert JM, Malick A, Coen DM, Martuza RL. Reduction and elimination of encephalitis in an experimental glioma therapy model with attenuated herpes simplex mutants that retain susceptibility to acyclovir. *Neurosurgery.* 1993;32:597–603.
146. Whitman ED, Tsung K, Paxson J, Norton JA. *In vitro* and *in vivo* kinetics of recombinant vaccinia virus cancer-gene therapy. *Surgery.* 1994;116:183–188.
147. Puhlmann M, Gnant M, Brown CK, Alexander HR, Bartlett DL. Thymidine kinase–deleted vaccinia virus expressing purine nucleoside phosphorylase as a vector for tumor-directed gene therapy. *Hum Gene Ther.* 1999;10:649–657.
148. Peplinski GR, Tsung K, Meko JB, Norton JA. Prevention of murine breast cancer by vaccination with tumor cells modified by cytokine-producing recombinant vaccinia viruses. *Ann Surg Oncol.* 1996;3:15–23.
149. Peplinski GR, Tsung K, Casey MJ, et al. *In vivo* murine tumor gene delivery and expression by systemic recombinant vaccinia virus encoding interleukin-1beta. *Cancer J Sci Am.* 1996;2:21.
150. Mastrangelo MJ, Maguire HC Jr, Eisenlohr LC, et al. Intratumoral recombinant GM-CSF–encoding virus as gene therapy in patients with cutaneous melanoma. *Cancer Gene Ther.* 1999;6:409–422.
151. Gnant MF, Puhlmann M, Alexander HR Jr, Bartlett DL. Systemic administration of a recombinant vaccinia virus

expressing the cytosine deaminase gene and subsequent treatment with 5-fluorocytosine leads to tumor-specific gene expression and prolongation of survival in mice. *Cancer Res.* 1999;59:3396–3403.
152. Mukherjee S, Haenel T, Himbeck R, et al. Replication-restricted vaccinia as a cytokine gene therapy vector in cancer: persistent transgene expression despite antibody generation. *Cancer Gene Ther.* 2000;7:663–670.
153. Hodge JW, Sabzevari H, Yafal AG, et al. A triad of costimulatory molecules synergize to amplify T-cell activation. *Cancer Res.* 1999;59:5800–5807.
154. McCart JA, Ward JM, Lee J, et al. Systemic cancer therapy with a tumor-selective vaccinia virus mutant lacking thymidine kinase and vaccinia growth factor genes. *Cancer Res.* 2001;61:8751–8757.
155. Norman KL, Coffey MC, Hirasawa K, et al. Reovirus oncolysis of human breast cancer. *Hum Gene Ther.* 2002;13: 641–652.
156. Gromeier M, Lachmann S, Rosenfeld MR, Gutin PH, Wimmer E. Intergeneric poliovirus recombinants for the treatment of malignant glioma. *Proc Natl Acad Sci USA.* 2000;97:6803–6808.
157. Balachandran S, Porosnicu M, Barber GN. Oncolytic activity of vesicular stomatitis virus is effective against tumors exhibiting aberrant p53, Ras, or myc function and involves the induction of apoptosis. *J Virol.* 2001;75:3474–3749.
158. Peng KW, TenEcyck CJ, Galanis E, et al. Intraperitoneal therapy of ovarian cancer using an engineered measles virus. *Cancer Res.* 2002;62: 4656–4662.
159. Peng KW, Ahmann GJ, Pham L, et al. Systemic therapy of myeloma xenografts by an attenuated measles virus. *Blood.* 2001;98:2002–2007.